Standard Operating Procedure for “Closed Nucleus Colony Laboratory Study”

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Queen caging

1. Find 6 empty drawn out black Pierco or wooden frames.
2. Place the frame on a flat surface with the top bar facing away from you.
3. Lay the wooden T-shaped pin-guide on top of the frame so that the top of the T is against the inside of the left-hand side of the frame.
4. Place a red map pin in the hole on the left and a green map pin in the hole on the right.
5. Lift the T-shape off of the frame and place a yellow pin in the cell diagonal from the red pin on the lower right-hand side. Repeat with a blue pin diagonal from the green pin.
6. Prepare top and bottom barcodes for each hive. Each frame should have two barcodes; one for the top of the frame and one for the bottom of the frame. The arrows on both the top and bottom barcodes should be pointing towards the pins. The “bottom” barcodes should be placed on the top bar with the arrows pointing toward the comb and the ‘top’ barcodes should be placed on the bottom bar of the frame with the arrows facing up. Attach the barcode using hot glue. Staples can be used on wooden frames but make sure not to staple areas with barcodes or writing.
7. Once a queen is found in a colony carefully place her on the pinned frame and mark her on the thorax with a paint pen, if needed. Put the cage over the queen and press down so that there are no holes between the wax and the cage. Make sure to leave enough space for the queen to move easily under the cage. Return frame to the host colony near frames of brood and leave enough space between the cage and the frame next to it for worker bees to get through.

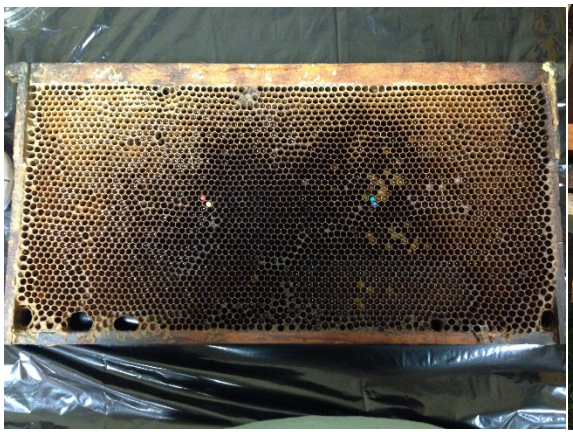

Figure 1: Egg frame preparation

Figure 2: Caging queen

Preparing pollen frames

1. Locate six empty drawn frames
2. Measure 90 grams of pollen and pour it into the Ninja food processor.

3. Add 5ml of distilled water and 5ml of diluted pesticide to the food processor and blend for thirty seconds. After 15 second scrape the sides of the food processor into order to blend pollen more thoroughly.
4. Take a 1-2 gram sample of pollen from each treatment used and put it in the freezer.
5. Weigh the pollen frame before and after the addition of pollen.
6. Push pollen into one side of the frame by pouring the contents of the pollen onto the frame and rolling it into the empty cells with your hands. Use gloves to do this and make sure to switch gloves between treatment groups.
7. Spray the frame a few times with 1:1 sugar solution to help the pollen stay in the frame.
8. Photograph pollen frames using only camera 2 on the Red Carpet. Label the frame using pieces of paper. Zoom the camera to the appropriate field of view and take a photo by pressing the shutter-release button on the camera.
9. Nail a queen pheromone strip (BeeBoost, Pherotech) attached to a zip tie to the top of the pollen frame.

Figure 3: Pollen frame preparation

Collecting egg frames and nurse bees

1. Return to host colonies with caged queens 24 hours after caging and locate the queen.
2. Remove cage and release queen into the bottom hive box.
3. Shake two frames of nurse bees from upper boxes into bulk nurse bee box with a frame containing multiple pieces of queen pheromone frame. If hive is not strong take nurse bees from another hive but make sure not to shake the queen into the nurse bee box.
4. Use a spray bottle with 1:1 sugar solution to keep nurse bees from flying out of the box. Leave box in a shady area until the nurse bees are ready to be added to the hives.
5. Weigh egg frames and photograph them using the Red Carpet.
6. Place each frame into a closed plastic nucleus colony (BeeBrief) with respective pollen frame and empty division board feeder with #8 mesh screen inside..
7. Face eggs towards pollen.

8. Label nucleus hive box and lid with hive number, treatment, and date.
9. Weigh each empty nucleus hive box.
10. Add two scoops of nurse bees (approximately 1 pound or 350g of bees) into each hive box using yellow milk jug scoop.
11. Weigh hive boxes after the addition of nurse bees.
12. Take hives to the storage building and place a mason jar feeder on top of the screen lid of each hive. Make sure the holes of the feeder are directly over the division feeder in the hive so that leaking sugar solution will fall into the feeder below.
13. Mason jar feeders (1 qt.) should contain 250ml of 1:1 sugar solution and be replaced every 3-4 days.

Figure 4: Collecting nurse bees.

Moving egg frames to incubating colony

1. Check control nuc to see if at least 75% of brood are capped.
2. If brood on the control frame are capped transfer all nuc boxes to a location where remaining bees can be released.
3. Take egg and pollen frames and leave nuc boxes open to allow remaining bees to fly out.
4. Photograph and weigh egg and pollen frames using The Red Carpet.
5. Take a 1-2g sample of pollen from each pollen frame and freeze it.
6. Put egg frames into incubating colony facing other brood frames.
7. Record which incubating colony each egg frame is located in.
8. Measure dead bees remaining in each nuc boxes.

Emergence

1. Take frames of brood out incubating colonies the day before emergence.
2. Weigh and photograph frames using the Red Carpet
3. Place each frame into a Rubbermaid cooler and place into incubator at 34˚C and 80% RH.
4. Weigh emerging bees and frame each day.
5. Take photo of frames using the Red Carpet.